$_{\Delta}\bar{\mu}H^{+}$-sensing in taxis of *Vibrio harveyi*

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1. INTRODUCTION

The study of $_{\Delta}\bar{\mu}H^{+}$-sensing in bacteria began with the findings of Ordal and Goldman [1,2] which proved that uncouplers of oxidative phosphorylation were potent repellents for *Bacillus subtilis*. On the basis of various experimental data, a hypothesis of a specific $_{\Delta}\bar{\mu}H^{+}$-receptor, a protometer, was suggested [3]. It was proposed that it governed unspecific repellent taxis, aerotaxis and phototaxis. The sensing of $_{\Delta}\bar{\mu}H^{+}$ was suggested to be a basis for uncoupler taxis in a study of *B. subtilis* taxis in [4].

Here, we report that *Vibrio harveyi* 392 is supersensitive to uncouplers, responding to 10^{-11} M FCCP. This fact, as well as the finding of increased sensitivity to uncoupler under anaerobic conditions, strongly supports the hypothesis of a protometer.

2. MATERIALS AND METHODS

Vibrio harveyi 392, a marine luminescent bacterium, was kindly provided by Professor Hastings of Harvard University. Bacteria were grown aerobically with rotary shaking at 28°C to a density of 0.5–0.7 ($1 = 6 \times 10^{8}$ cells/ml, $D_{660\,nm}$) which corresponded to the mid-log phase. The growth medium contained in 1 l: 5 g yeast extract (Bacto), 5 g tryptone (Bacto), 30 g NaCl, 1 g KH_2PO_4, 7 g $Na_2HPO_4 \cdot 12\,H_2O$ and 0.25 g $MgSO_4 \cdot 7\,H_2O$ (pH 7.1). Cells were harvested by centrifugation and washed twice in the salts portion of the growth medium additionally containing 0.03% glycerol as an oxidizable substrate (hereafter referred to as 'buffer'). Cells resuspended in buffer to 5×10^{6}/ml were 90% motile.

FCCP and CCCP were from Sigma, DNP and indole from Serva. All other chemicals used were of reagent grade.

Taxis was registered microscopically, essentially as in [5]. The monoflagellar *V. harveyi* 392 periodically spontaneously reverses its direction of movement, the addition of a repellent causes oscillatory reversals, while the addition of an attractant suppresses reversal. The time of recovery in relation to the unstimulated rate of reversals (adaptation time) was determined by a stopwatch in 12–15 independent experiments.

Abbreviations: FCCP, carbonyl cyanide 4-trifluromethoxyphenylhydrazone; CCCP, carbonyl cyanide 3-chlorophenylhydrazone; DNP, 1,4-dinitrophenol

3. RESULTS

Uncouplers appeared to be potent repellents for *V. harveyi* 392, having an extremely low threshold concentration and causing prolonged oscillatory reversals of cells in higher concentrations (table 1). Bacteria fully adapted to added uncouplers with restoration of the frequency of reversals in relation to the unstimulated rate. The maximal tactic response was observed upon the addition of 10^{-8} M CCCP which caused a prolonged, 110 ± 3 s oscillatory reversing movement. At this concentration, CCCP slightly inhibited the rate of translational movement.

Acetate and indole, which are repellents of *E. coli* [6], also appeared to repel *V. harveyi* (table 1). In *E. coli*, indole is sensed by a specific, although as yet undetermined receptor [6], while acetate acts by decreasing the cytoplasmic pH which is registered by pH-taxis [7,8]. With *V. harveyi*, however, saturation of the taxis response to indole at elevated con-

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Table 1

The response of *V. harveyi* 392 to various repellents

Additions	Conc. (M)	Adaption time (s)
FCCP	10^{-11}	10±2
FCCP	10^{-10}	25±3
FCCP	10^{-9}	55±2
CCCP	10^{-10}	40±2
CCCP	10^{-9}	70±3
CCCP	10^{-8}	110±3
DNP	10^{-6}	73±4
DNP	10^{-8}	35±2
Acetate	10^{-6}	45±3
Acetate	10^{-7}	21±1
Indole	10^{-6}	65±2
Indole	10^{-8}	27±2
Buffer	5 μl	–

Bacteria from a stock suspension in buffer (5×10^6 cells/ml) were diluted to 5×10^5 cells/ml and added in 5 μl drops onto a microscope slide. The reagent dissolved in buffer was added in an equal volume; the preparation was then sealed with a coverslip and immediately observed in phase contrast microscope with a 40 × objective. The results are the mean of 12–15 independent measurements. The concentration of ethanol in the probes with FCCP and CCCP was < 0.0001%. Bacteria produced no response to this concentration of ethanol dissolved in buffer.

Table 2

The effect of KCN on the sensitivity of taxis towards attractants and repellents

Additions	Conc. (M)	Adaption time (s)	
		− KCN	+ KCN
FCCP	10^{-10}	25±3	70±4
CCCP	10^{-10}	40±2	90±3
DNP	10^{-8}	35±2	63±2
Acetate	10^{-7}	21±1	34±4
Indole	10^{-8}	27±2	36±2
Valine	10^{-5}	32±2	31±4
Isoleucine	10^{-5}	42±1	41±2
Threonine	10^{-7}	30±3	31±2

Conditions were essentially as given in legend to table 1. Cells were incubated for 10 min with 5×10^{-4} M KCN

centrations was not observed since 10^{-4} M indole arrested movement, most probably due to a toxic side-effect; nor could the taxis response be saturated by acetate. This latter result was predictable, in view of its action in decreasing the cytoplasmic pH.

Among amino acids, threonine caused a transient suppression of tumblings, acting as an attractant, while the hydrophobic amino acids valine and isoleucine were found to repel the cells. It was reported in [6] that valine and isoleucine repelled *Escherichia coli* cells. In high concentrations, valine did not impair motility. All the reported repellents were tested for their ability to cause a repelling action in the spatial gradient assay of [6] (repellent in plug method). The substance tested appeared to efficiently repel bacteria from an area containing the repellent (not shown). The sensing of these 4 types of repellent and the corresponding attractant amino acid could alternatively be governed either by a protometer, or by specific chemoreceptors (or additionally by pH-taxis in the case of acetate). To discriminate between these alternatives, the above effectors were added in the presence or absence of 5×10^{-4} M KCN, a concentration that inhibited respiration by 50% (not shown). An uncoupling agent could be expected to cause a larger decrease in $\Delta\bar{\mu}H^+$ in the absence of an actively operating redox chain than in its presence, thus increasing the behavioural response governed by a protometer. In the case of a specific chemoeffector or if $\Delta\bar{\mu}H^+$-sensing were absent, inhibition of the redox chain would not result in increased taxis sensitivity.

As the data in table 2 show, the response to 10^{-10} M FCCP increased considerably in the presence of KCN reaching 350% of the control value. The responses to CCCP and DNP were also increased by KCN which had less effect on sensitivity to acetate and still less effect on sensitivity to indole. The responses to both attractant and repellent amino acids were unaffected by KCN.

4. DISCUSSION

Vibrio harveyi 392 a monoflagellar marine bacterium, appears to be extremely sensitive to uncouplers, having a threshold taxis response to FCCP at as low as 10^{-11} M. The most sensitive response to an uncoupler (FCCP) to date was reported in a study of *B. subtilis*, its threshold concentration (10^{-8} M) being 3 orders of magnitude higher than

in our study [2]. This very low active uncoupler concentration suggests that the behavioural response is mediated by $_{\Delta}\bar{\mu}H^+$-sensing, since it is difficult to imagine a specific binding receptor for FCCP that operates at an efficiency of several orders of magnitude higher than that of an attractant receptor. At the same time, an uncoupler may cause a general decrease of $_{\Delta}\bar{\mu}H^+$ that will in turn be registered by a protometer.

We have reported that the addition of KCN markedly increased the sensitivity of *H. halobium* to phototaxis [9]. The same approach was used here: supposing that changes in $_{\Delta}\bar{\mu}H^+$ brought about by an uncoupler would be greater in the absence of active respiration, it was expected that KCN would increase the sensitivity to uncouplers. Indeed KCN greatly increased the adaptation time to uncoupler. Acetate taxis, which is probably mediated by pH-sensing [7,8], may be sensitized by KCN. This precludes alkalization of the cytoplasm that would normally be achieved by respiration. The effect of KCN on acetate taxis, however, is less pronounced than on uncoupler sensing, suggesting that the uncoupler does not act through pH-taxis but by means of $_{\Delta}\bar{\mu}H^+$-sensing. A slight activation of indole taxis by KCN suggests that sensing is in part due to a specific receptor, and in part to $_{\Delta}\bar{\mu}H^+$-sensing, since high concentrations of indole are toxic and probably decrease $_{\Delta}\bar{\mu}H^+$. The response to attracting and repelling amino acids was independent of KCN, indicating that these effectors are sensed by a specific chemoreceptor.

The very high sensitivity of *V. harveyi* to uncouplers makes it a promising organism for further studies of $_{\Delta}\bar{\mu}H^+$-sensing.

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